Compound Light Microscope and Other Different Microscopes

24

24.1 Light

Light is an electromagnetic wave with specific amplitude and wavelength. The distance between the two successive peaks of the light wave indicates the wavelength of the light. The distance between the crest to the horizontal axis is known as amplitude. The time to travel one crest to successive next crest is known as period. The frequency of light wave is indicated by the number of the wave cycle per unit of time (Fig. 24.1). The visible light remains within the wavelength of 400–750 nm. This is a very tiny fraction of the electromagnetic spectrum (the radio waves are 10^4 m and ϒ rays 10^{-10} m). The wavelength of the red light is 750 nm (1 nm = 10^{-9} m) and that of blue light is 400 nm (Fig. 24.2). Ordinary white light is comprised of all the colours. The dominant wavelength of the source of the light determines its colour.

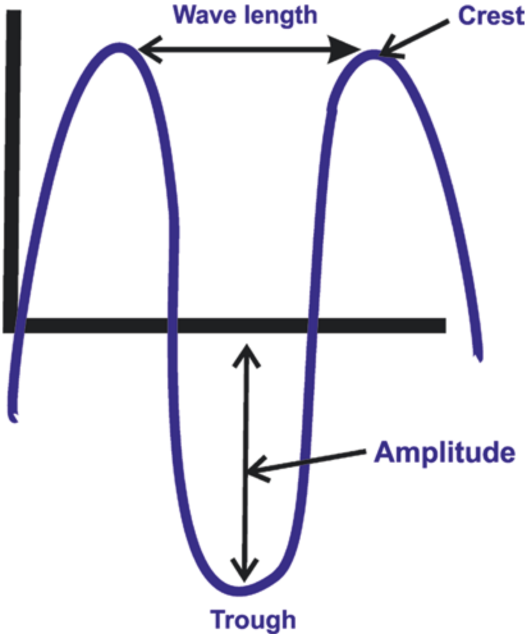

Fig. 24.1 Schematic diagram showing wave of light. The distance between the two successive peaks of the light wave indicates the wavelength of the light

24.2 Colours

There are different modules to express the colour.

Hue Saturation Intensity (HSI) This is one of the common modules to express colour. Hue indicates the basic colour such as blue, green or red. The saturation means how deep is the colour that means whether the colour is pale or dark. Intensity measures the brightness of the colour.

Red Green Blue (RGB) There are three primary colours: red, green and blue. The other colours that we perceive are the mixture of these colours such as admixture of red and green colour will produce yellow colour. The percentage

© Springer Nature Singapore Pte Ltd. 2018
P. Dey, *Basic and Advanced Laboratory Techniques in Histopathology and Cytology*,
https://doi.org/10.1007/978-981-10-8252-8_24

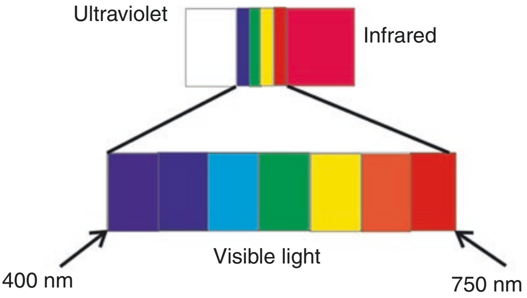

Fig. 24.2 Visible spectrum of light is 400–750 nm wavelength of light

expression of these three primary colours represents the colour of that particular object.

CYM The subtraction of one or more primary colour from the white light produces three other secondary colours: cyan (C), yellow (Y) and magenta (M). Therefore this CYM colour model is known as primary subtractive colours. This model is mainly used in optical filter and printing.

Perception of Colour Cone cells are the photoreceptor cells of the retina. They are exclusively related with the colour perception and consist of only 5% cells. The cone cells are mostly concentrated in the fovea centralis of the retina. We perceive white colour when the light equally stimulates all the three types of cone cells that means red, green and blue. If the light stimulates only the red-sensitive pigments of the cone cells, then we perceive red colour. Similarly if the light stimulates both red and blue pigments of the cone cells, then we perceive magenta colour.

24.3 Image Generation and Human Vision

Eye Is a Biological Camera The image of the object is formed on the retina of the eye (Fig. 24.3). At first the light rays from the object pass through the cornea, aqueous humour, lens and vitreous humour, and finally the light stimulates the photoreceptor cells of the retina. The initial image in the retina is an inverted image of the object. The photoreceptor cells of the retina pass the electrical signal through the optic nerve to the brain. Human brain corrects the inverted image to the normal erect image.

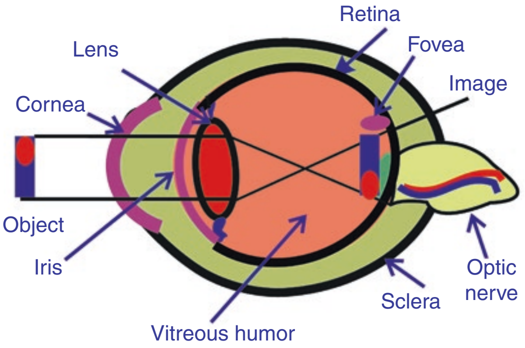

Fig. 24.3 Image formation in the human eye is highlighted in this schematic diagram. The image of the object is formed on the retina of the eye. The initial image in the retina is an inverted image of the object. The photoreceptor cells of the retina pass the electrical signal through the optic nerve to the brain. The human brain corrects the inverted image to the normal erect image

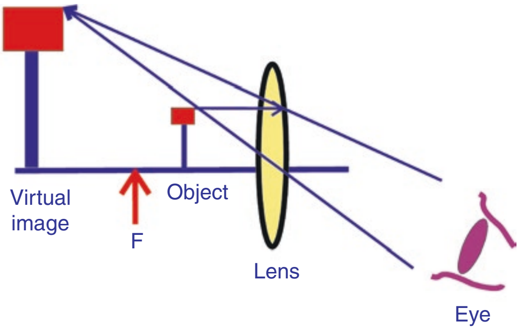

Fig. 24.4 Schematic diagram showing the formation of image by a convex lens. If the object is placed within the focal length of the lens, then we see a virtual enlarged image formed in the same side of the lens

Image Formation by Simple Lens The image formed in the retina of the human eye is non-magnified. The image of the object can be magnified if a convex lens is placed between the object and eye and appropriately focused (Fig. 24.4). If the object is placed within the focal length of the lens, then we see a virtual enlarged image formed in the same side of the lens. The focal length of the lens is defined as the distance between the optical centre of the lens and the focal centre where the image is perfectly focused.

Light Microscope [1] The light microscope deals with the visible light and so it is named as light microscope. The predominate three functions of this microscope are:

- Magnification
- Resolution
- Contrast

Magnification The word magnification indicates the enlargement of the image of object of interest. The objective and the eyepiece take part in the magnification of the image of the object. The first magnification takes place by the objective. The power of magnification is written on the wall of the objectives of the microscope. Normally the power of magnification varies from 4 times to 100 times in ordinary biological light microscope. The second magnification is done by the eyepiece, and so the final magnification is equal to the first magnification done by objective multiplied by the second magnification done by the eyepiece.

$$M = M_1 \times M_2$$

M = Final magnification
M_1 = Linear magnification by objectives
M_2 = Linear magnification by eyepiece

The final magnification of the different objectives is shown in Table 24.1.

Resolution The term resolution means the ability of the microscope to distinguish two closely spaced objects. If we put two objects at a distance, then we are immediately able to identify them as two separate entities. However, the more and more they are kept close together, the more it is difficult to recognize them as two separate entities. At a certain distance, it may be impossible to distinguish these two objects, and they may look as one object. The minimum distance where the two objects can be distinguished separately is known as resolution capacity (D) of the microscope. The resolution of the microscope is dependent on two factors:

1. The wavelength of the light
2. The maximum angle of light that can be obtained by the objective lens from the object

Table 24.1 Magnification of the microscope using different objectives

Objectives	Eyepiece	Final magnification
2×	10×	20×
4×	10×	40×
10×	10×	100×
20×	10×	200×
40×	10×	400×
100×	10×	1000×

Numerical Aperture (Fig. 24.5) Numerical aperture (NA) is related with the light gathering power of the objective. In fact the resolution power of the microscope is largely dependent on the NA of the objective, and it is represented by the equation below:

$$D = \frac{0.61\sigma}{\text{NA}}$$

D = resolution
Sigma (σ) = the wavelength of light
NA = numerical aperture
Numerical aperture is calculated as:
NA = n × Sin μ
n = refractive index of air (it is 1)
μ = half of the angular aperture of the objective

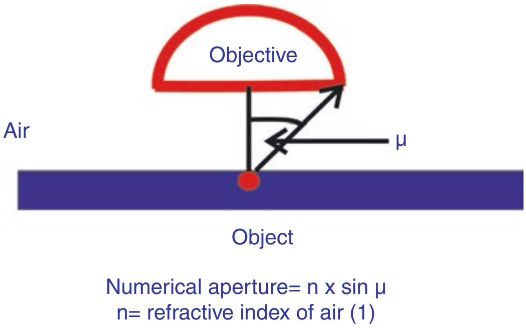

Fig. 24.5 Numerical aperture of the microscope is calculated as mentioned in the diagram. Numerical aperture is related with the light gathering power of the objective

Contrast The contrast of the microscope means the ability to detect an object from the background material. If we want to know the details of an object, then we need to have the difference of intensity of the colour between the object and the background material.

Image formation by the light microscope (Fig. 24.6)

The compound light microscope works in the same way as described in the magnification by convex lens (Box 24.1). The microscope is composed of bunches of properly placed lens that magnifies the image of the object in severalfolds. There are two sets of lens: objectives and the eyepiece. The object remains in between the condenser and the objective. The condenser condenses light through the object. The lens of the objective has short focal length and generates a magnified real image within the body tube of the microscope. Subsequently lens in the eyepiece further magnifies this real image, and a virtual more magnified image is formed approximately 25 cm distance from the eye in the same side of the lens near the object in the stage.

Box 24.1: Image Formation in Microscope

- First image is formed by the magnification by the objective.
- First image:
  - Real
  - Magnified away from the lens in the same side
  - Formed in the body of the microscope
- Second image is formed by the eyepiece which is the magnification of the first image.
- Second image:
  - Virtual
  - Highly magnified
  - Formed 25 cm away from the eye
  - Location: Between the stage and condenser

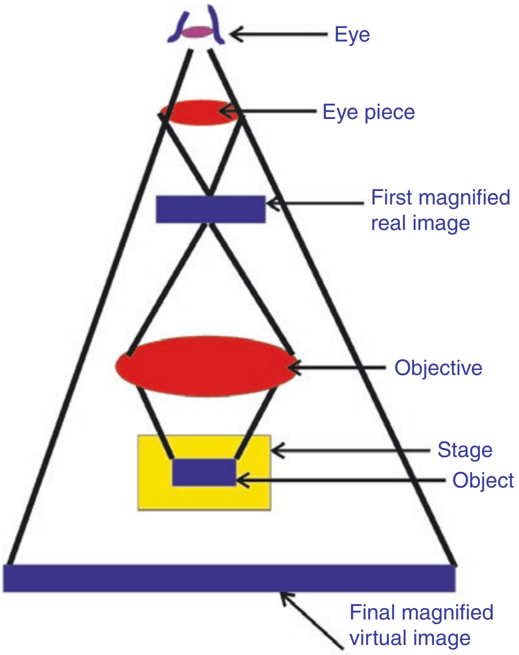

Fig. 24.6 Schematic diagram showing the formation of image by the light microscope. The lens of the objective has short focal length and generates a magnified real image within the body tube of the microscope. Subsequently lens in the eyepiece further magnifies this real image, and a virtual more magnified image is formed in the same side of the lens near the object in the stage

24.4 Anatomical Components of a Light Microscope (Fig. 24.7)

Base The base is the supporting platform of the microscope.

Stage The stage is the moveable component of the microscope. The slide is placed on the stage, and then the stage is moved up and down to focus the object. The slide on the stage is also moved in different directions.

Coarse and Fine Adjustment Knobs These are two knobs placed in both sides of the body of the microscope. These knobs are rotating and helping in the focusing of the objectives by moving it up and down.

Tube The tube is the connecting part between the objectives and eyepiece.

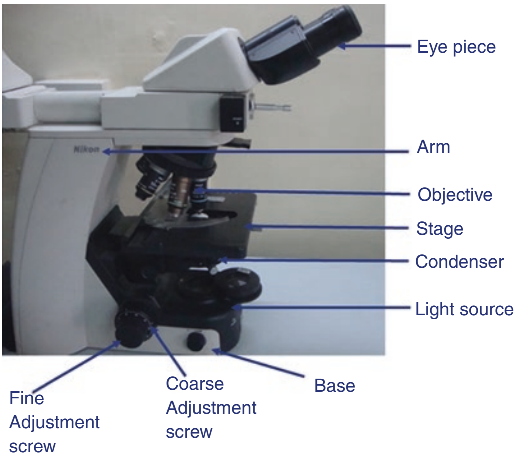

Fig. 24.7 Simple light microscope and its parts

Arm Arm connects the base of the microscope with the tube.

Revolving Nosepiece The various objectives are placed in the resolving nosepiece.

Diaphragm The diaphragm is located below the stage and helps to control the amount of light on the object.

Light Source The source of light is located on the base of the microscope; the light travels directly from the source to the object and then through different lenses.

24.5 Optical Components [2, 3]

The following parts are the constituents of the optical part of the light microscope:

1. *Condensers*: The condenser of the microscope accumulates light from its source and then concentrates the light on the object as a cone of light so that the maximum light passes through the object to get better resolution. The object is illuminated by parallel beam of light with uniform intensity. The condenser aperture diaphragm helps in controlling the diameter of the light beam. With the help of a screw, the condenser can be adjusted for the proper focusing of the cone of light. However once the objective is changed, then the position of the condenser has to be readjusted.
2. *Objectives*: Each microscope contains a set of multiple objectives attached with the rotating nosepiece. The circular ring of the objective represents the magnification power of objective, and the different objectives have different coloured rings on it (Fig. 24.8). The various information such as lateral magnification, numerical aperture, etc. are engraved on the objective. Presently the objectives are designed as infinitely corrected. The following are the different types of objectives:
   - *Achromat*: The achromatic objectives contain two doublets and a single front lens. They are relatively cheaper and quality suitable for the low magnification images. However they are not very satisfactory for higher magnification.
   - *Fluorite or semiapochromat lens*: Fluorite type of objective lens is made of fluorspar or newer synthetic substitutes and provides good level of colour correction. They are highly transparent and give high contrast. Therefore this type of objectives is suitable for immunofluorescence microscopy and polarized microscopy.
   - *Apochromatic*: These are relatively costly lens and give very good correction. The apochromatic objectives are the most suitable for colour photography as the chromatic aberration is nearly zero in such lens.

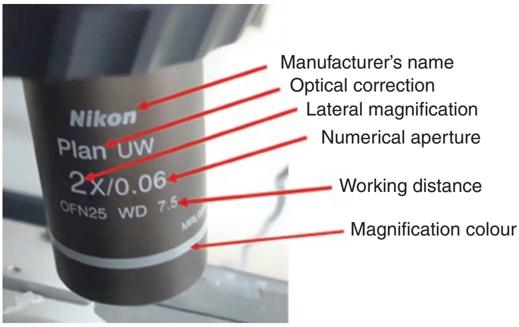

Fig. 24.8 Objective of the compound microscope. Note the basic information engraved on it

24.5.1 The Major Aberrations of the Lens

The spherical lens of the microscope may have several optical faults or aberrations. Image distortion occurs due to these various optical aberrations. The common aberrations are:

Chromatic Aberration (Fig. 24.9a) The chromatic aberration occurs due to the different refractions of different wavelengths of light. Blue light is bended more than the red light. Therefore there are different locations of images for the different wavelengths of the colour, and overall the image becomes distorted. More disastrous is the different magnifications of the different colours of images as they are located in different distances from the lens.

Solution: The solution of the chromatic aberration is to use the different components of the lens with different dispersing properties for the different colours so that the final images overlap in the same location.

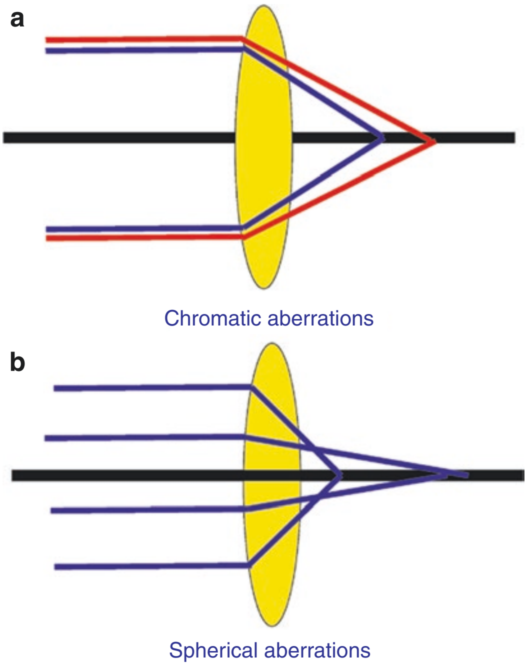

Fig. 24.9 (**a**) Principle of chromatic aberrations is explained in this schematic diagram. The chromatic aberration occurs due to the different refractions of different wavelengths of light. (**b**) Principle of spherical aberrations is explained in this schematic diagram. This is due to different bending capacities of the central and peripheral part of the spherical lens

Spherical Aberration (Fig. 24.9b) The spherical aberrations occur when spherical lens is used for magnification in the microscope. The cause of spherical aberrations is the different bending capacities of the central and peripheral part of the spherical lens. The incident rays of light that pass through the peripheral part of lens bend more than the incident rays that pass through the centre of the lens. Therefore in case of spherical aberration, the images are formed in different focal planes, and the image may be blurred in the periphery.

Solution: The solution of spherical aberrations is to apply a collection of different thickness of positive and negative lens.

Astigmatism This is an off-axis aberration, and it occurs due to the defect in the manufacture of the proper curvature of the lens or defective placement of the lens. Here the rays of light from the object going through the horizontal and vertical diameters of the lens focus in two separate focal planes. The astigmatism increases when the object is more away from the optic axis.

Solution: Good quality lens and proper placement of the lens

Curvature of Field In this aberration the image is curved instead of flat, and the whole image cannot be focused in a single flat plane.

Solution: The curvature of field can be corrected by proper designing of the lens of the objective.

3. *Eyepiece*: Eyepiece or the ocular lens magnifies the real image produced by the objective in the body tube of the microscope. Ocular lens generates the final image which is a virtual image and 25 cm away from the eye. The specification of the ocular lens is usually engraved on the lens barrel.

Nowadays most of the eyepieces provide magnification in the range of 10× to 20×. The ocular lens or eyepiece can be focusable or non-focusable. Eyepieces may be of two types:

Negative Eyepiece It contains internal diaphragm between the lenses of the ocularis (eyepiece). The simple negative eyepiece is also known as Huygenian eyepiece which is commonly used in routine light microscope.

Positive Eyepiece It contains internal diaphragm below the lens of the ocularis (eyepiece). This type of eyepiece is also called as Ramsden eyepiece.

24.6 How to Take Care and Handle Your Microscope

The basic care of microscope is highlighted in Box 24.2.

- *Starting*: Connect the plug of the microscope with recommended electrical power supply.
- *Focus*: Focus gently and do not be fast. During the focusing of the object, please take care so that the objectives do not touch the slide and break it.
- *Bulb:* The bulb of the microscope is costly so please turn off the illumination when the microscope is not used.
- *Transport*: Grasp the arm of microscope by one hand and support the base by the other hand. Keep the base of the microscope parallel to the ground so that the optical systems are not displaced or fall down. Never swing a microscope.
- *Touch*: Never touch the lens of the microscope by hand or any body parts because the normally producing oil in the body may blur the lens.
- *Cleaning*: Clean the lens of the microscope only by lens paper. Do not use any toilet paper or towel paper as they may scratch the lens. Do not use distilled water or any sorts of organic solvent as they may dissolve the coating of the lens. It is important to note that excessive cleaning may be harmful to the lens. The best way to clean a lens is using compressed air.
- *Closing the work*: After finishing the work with the microscope, please take out the slide from the stage, keep the low power objective towards the stage and far away from the stage, disconnect the electric power source and cover the microscope by dust cover.

Box 24.2: Basic Care of Microscope

- *Plugging*: Connect the plug in the recommended electrical power supply.
- *Bulb*: Always put off the bulb when you do not use the microscope. Remember that the bulb is expensive.
- *Focus*:
  - Do not hurry.
  - Gently focus your object and take care that the objective should not touch the slide.
  - Always use lowest power of objective first.
- *Cleaning*: Clean the lens before and after you use. Clean only by lens paper. *Do not use* distilled water or any sorts of organic solvent.
- *Transport*:
  - Grasp the arm of microscope.
  - Keep the microscope parallel to the ground.
  - Keep it close to your body.
- *Damage*: Never try to repair any damage. Report to the authority.
- *After finishing work*:
  - Unplug.
  - Do not put any slide on stage. Clean the stage.
  - Adjust the nosepiece for the lowest power objective.
  - Cover the microscope to protect it from dust.

24.7 Other Types of Microscope

24.7.1 Dark-Field Microscope

In this type of microscope, a hollow beam of light is produced by blocking the central part of the beam of light (Fig. 24.10). The scattered light from the object passes through the objective to the eye, and the object is seen as bright in a dark background. Ordinary light microscope can be used as dark-field microscope by using dark-field block condenser. The central cylinder of light is obstructed, whereas the peripheral rim of light reaches to the object.

Use:

- The bacteria, spirochaetes, or fungi in suspension are better seen in dark-field microscope.
- Movement of the cells in culture medium is better seen in this microscope.

24.7.2 Bright-Field Microscope

This is the simple light microscope where the object is examined by attenuated light. No additional equipment is required for the bright-field microscopy. The diaphragm of the microscope should be fully opened, and light intensity should be kept at low. The light passes through the sample, and the denser area of the object absorbs part of the light. So the object looked dark in a bright background.

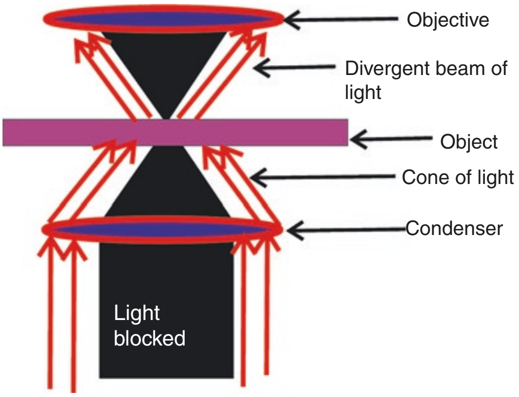

Fig. 24.10 Schematic diagram showing pathway of light in the dark-field microscope. A hollow beam of light is produced by blocking the central part of the beam of light

Use: Stained or natural specimen

Advantages:
• Very simple.
• No additional equipment's are needed.
• Object can be seen without staining.
Limitations:
• Low contrast
• Low resolution
• Low magnification

24.7.3 Phase Contrast Microscope

Principle In this microscope the objects having different refractive indices are identified as they produce different contrasts. The object with scattered light is identified from the illuminating background light. If the light passes through a transparent object, then due to the change of refractive index of the object, the pathway of light will be deviated slightly, and the light wave is retarded. In case of denser particle (higher refractive index) in the object, the deviation of light will be more, and the light wave is more retarded. This is known as phase difference. Usually these phase differences are invisible to us; however, the phase contrast microscope makes these changes significantly visible.

System (Fig. 24.11) In phase contrast microscope, a condenser annulus and modified objective with phase plate are used. Intense beam of light source is passed through the substaged condenser annulus which is located in the focal plane of the condenser. So a hollow cone of light is generated that can be controlled. Now this light passes through the objects/sample, and they are either deviated or undeviated depending on the refractive indices of the different structures of the object. Now both undeviated (central ray) and deviated light pass through the objective and are segregated by the phase plate that is located behind the focal plane of the objective.

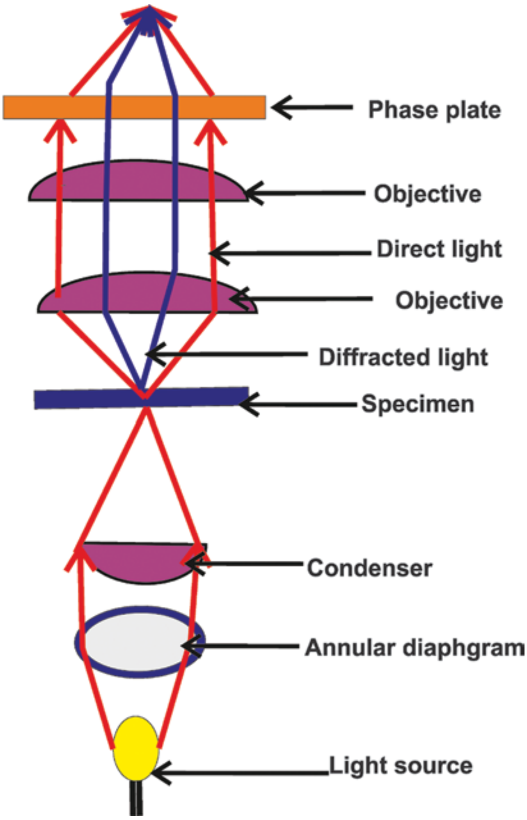

Fig. 24.11 Schematic diagram showing pathway of light in the phase contrast microscope. Here a condenser annulus and modified objective with phase plate are used. A hollow cone of light is generated that passes through the objects, and the lights are either deviated or undeviated depending on the refractive indices of the different structures of the object. The light passes through the objective and is further segregated by the phase plate behind the focal plane of the objective

Applications:

- This is used to see the living cells: shape, size, etc.
- Unstained protozoa or fungi are best seen in this microscope.
- Motility of the organism.

References

1. Wollman AJ, Nudd R, Hedlund EG, Leake MC. From animaculum to single molecules: 300 years of the light microscope. Open Biol. 2015;5(4):150019.
2. Wolf DE. The optics of microscope image formation. Methods Cell Biol. 2003;72:11–43.
3. Goodwin PC. A primer on the fundamental principles of light microscopy: optimizing magnification, resolution, and contrast. Mol Reprod Dev. 2015;82(7–8):502–7.